

**A Role for Nuclear Actin in HDAC 1 and 2 Regulation**

**Leonid A. Serebryanny, Christina M. Cruz and Primal de Lanerolle**

**From the Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL**

**SUPPLEMENTARY INFORMATION:**

## SUPPLEMENTARY FIGURE LEGENDS

**Figure S1:** Predicted binding partners of nuclear actin and HDAC 1 and 2. **(a)** Canonical protein composition of the common HDAC 1 and 2 chromatin regulatory complexes. Proteins that were identified by proteomics analysis of actin pulldowns are in bold faced black text on grey background. Unidentified proteins are in grey text on a white background. **(b)** Quantification of co-immunoprecipitation experiments as performed in **Figures 1a and b**. **(c)** Co-immunoprecipitation of DSP crosslinked HeLa nuclear extract using non-specific IgG, HDAC 1, and  $\beta$ -actin antibodies and blotted for HDAC 1 and  $\beta$ -actin. Protein-antibody bound beads were extracted in SDS buffer with 2-mercaptoethanol to reduce crosslinks before gel loading.

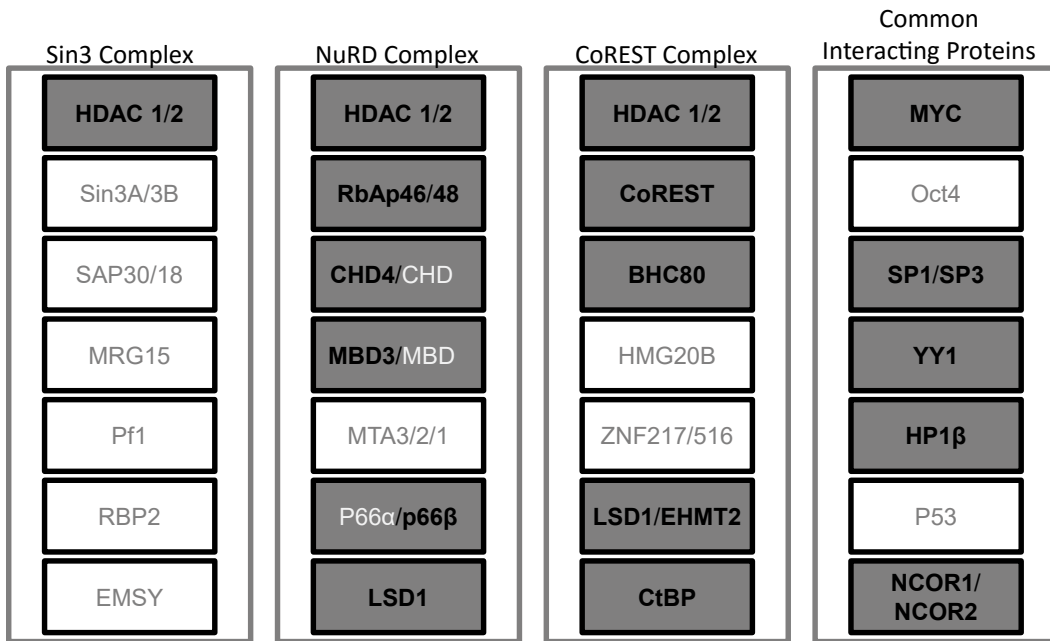
**Figure S2:** Nuclear actin alone does not directly inhibit HDAC 2 nor act as an HDAC 2 substrate. **(a)** HDAC activity of recombinant Flag-HDAC 2, purified non-muscle actin, substrate alone (blank), or all three incubated in G-actin or transcription buffer. Mean + SD, done in triplicate. **(b)** HeLa cells treated with 4  $\mu$ M TSA for 3 h, LatB 1  $\mu$ M 1h, or vehicle were extracted, immunoprecipitated with  $\beta$ -actin antibody, and immunoblotted for acetylated lysine residues. Similarly 50  $\mu$ g of purified non-muscle actin was incubated with and without 7  $\mu$ g of recombinant Flag-HDAC 2 and immunoblotted (right 2 lanes). No treatment showed visible changes in acetylation of the predicted actin band.

**Figure S3:** S14C NLS  $\beta$ -actin EYFP expression affects H4K16ac levels. **(a)** HeLa cells transfected with S14C NLS  $\beta$ -actin EYFP or R62D NLS  $\beta$ -actin EYFP and stained for HDAC1. **(b)** HeLa cells transfected with S14C NLS  $\beta$ -actin EYFP or R62D NLS  $\beta$ -actin EYFP or GFP as a control and blotted for the indicated proteins. **(c)** Representative images from **Figure 4f**. COS7 cells transfected with S14C NLS  $\beta$ -actin EYFP or R62D NLS  $\beta$ -actin EYFP (green) were fixed in methanol, digested with DNase I, and stained with DAPI (blue).

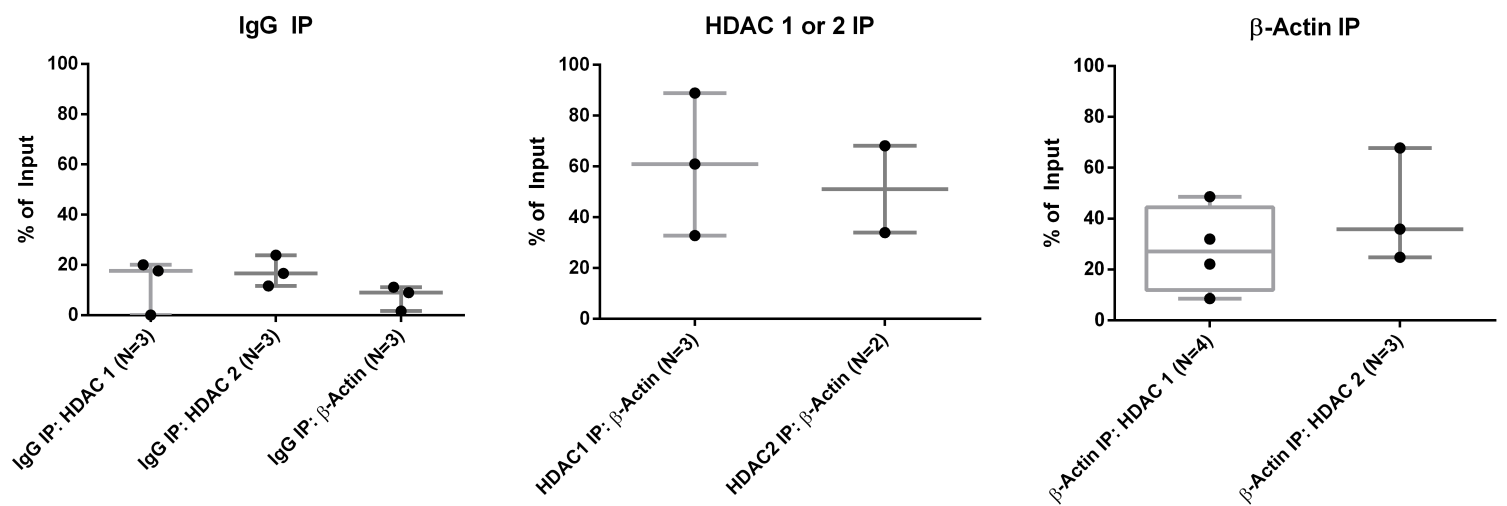
**Table S1:** Mass spectrometry results from actin-Sepharose bead pulldown and BSA-Sepharose bead pulldown. Protein identifications that appeared in the BSA-Sepharose pulldown were crossed out.

# Supplementary Figure S1

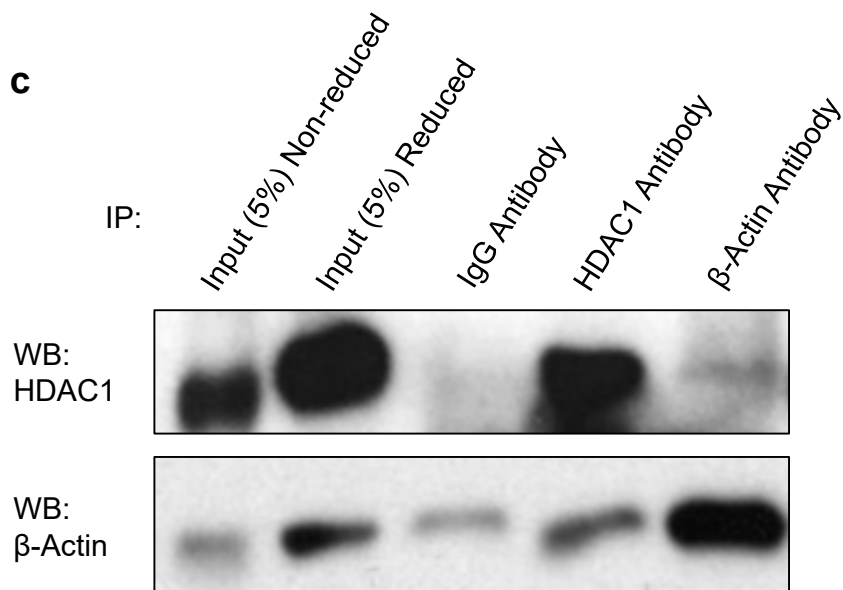
**a**



**b**

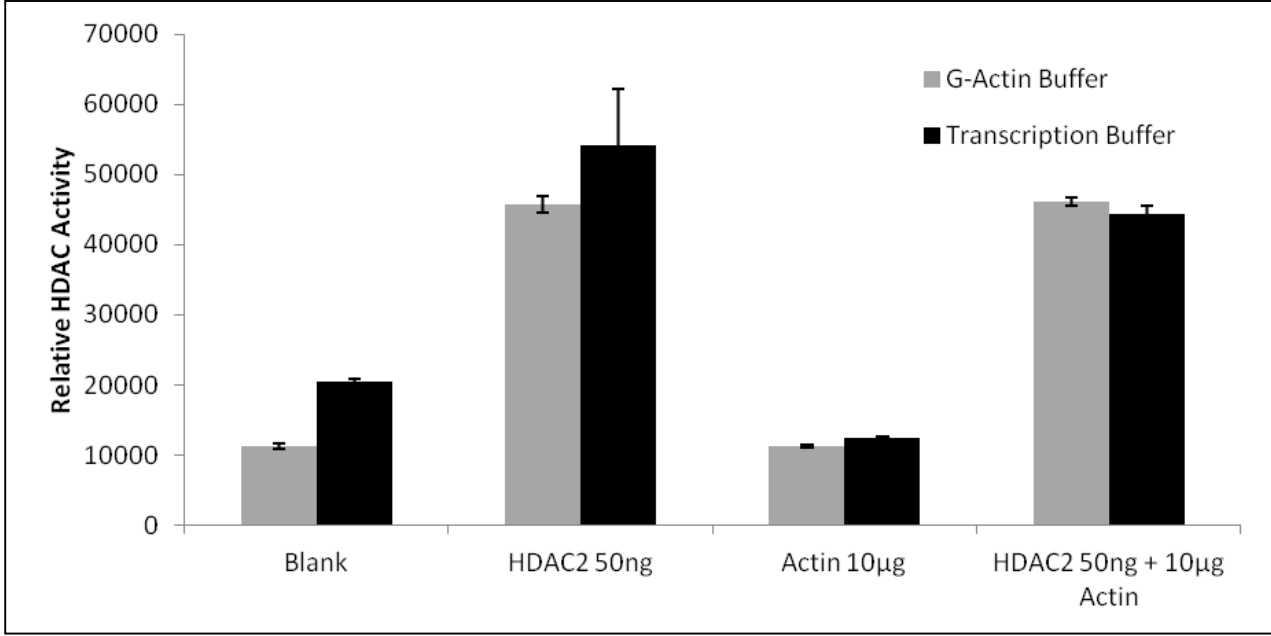


**c**



# Supplementary Figure S2

**a**



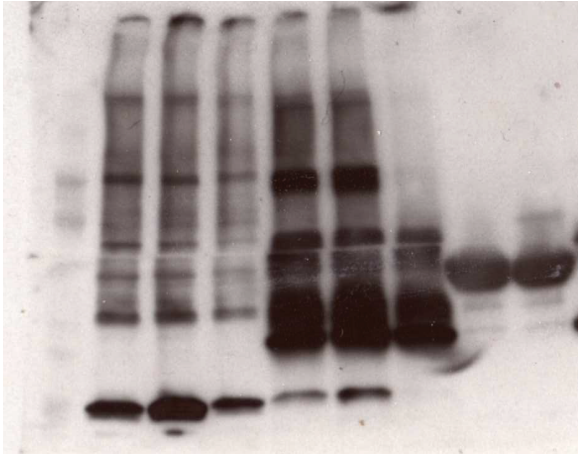
**b**

## Anti-Acetyl Lysine Blot

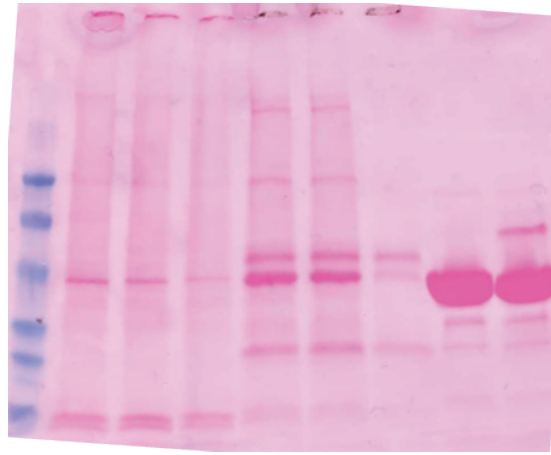
## Ponceau Stain

Non-treated Input  
TSA Treated Input  
LatB Treated Input  
Non-treated Actin IP  
TSA Treated Actin IP  
LatB Treated Actin IP  
50ug Purified Actin  
50ug Purified Actin + 7ug Purified HDAC2

Non-treated Input  
TSA Treated Input  
LatB Treated Input  
Non-treated Actin IP  
TSA Treated Actin IP  
LatB Treated Actin IP  
50ug Purified Actin  
50ug Purified Actin + 7ug Purified HDAC2



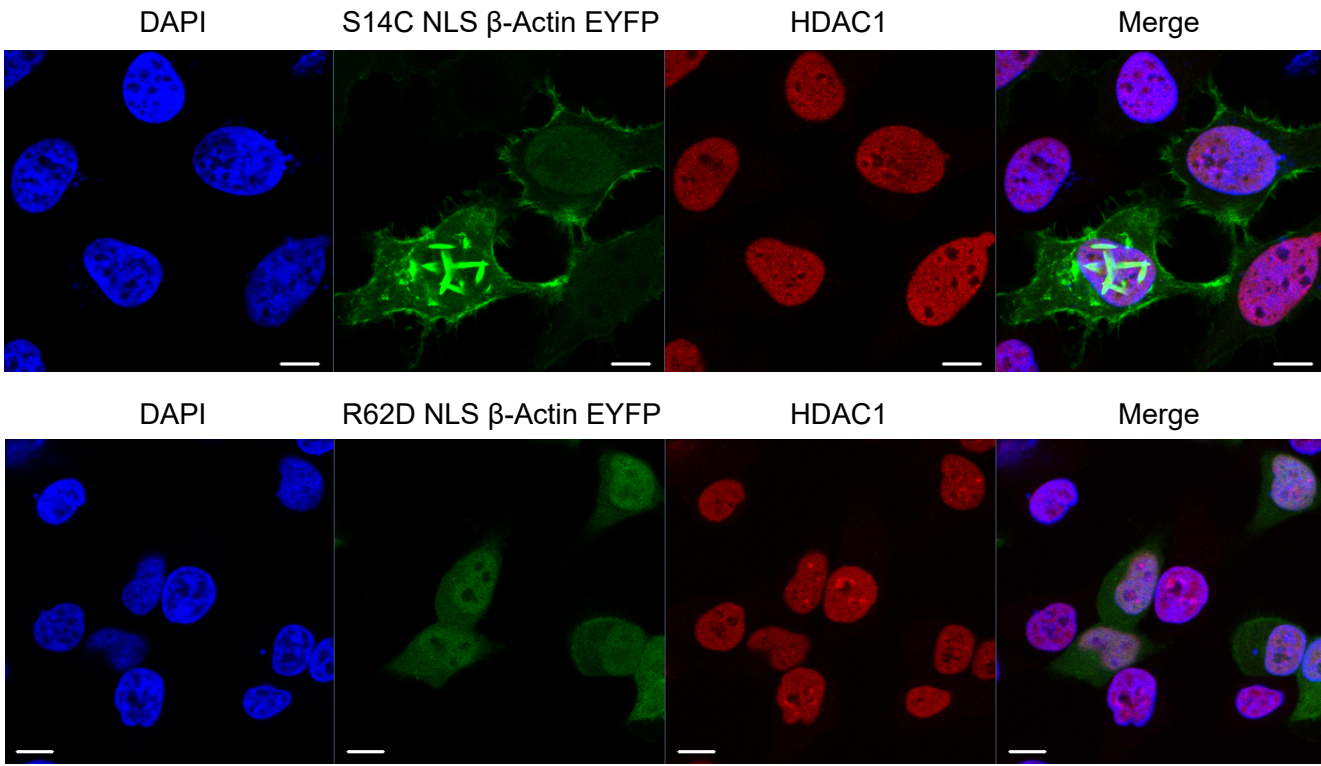
<- HDAC2  
<- IgG  
<- Actin



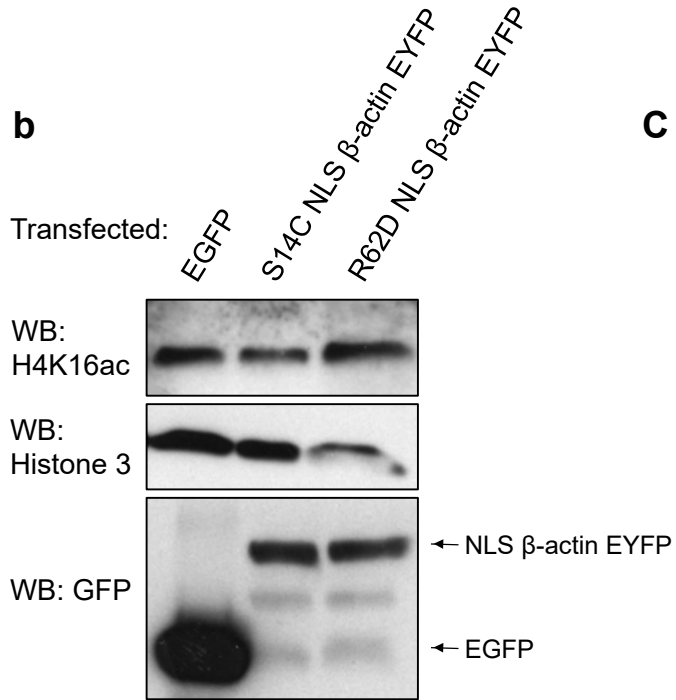
<- HDAC2  
<- IgG  
<- Actin

# Supplementary Figure S3

**a**



**b**



**c**

